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L10: Entry 32 of 37 File: USPT

DOCUMENT-IDENTIFIER: US 5627265 A
TITLE: Receptor for cell-binding domain of thrombospondins

Brief Summary Text (6):
In addition, TS plays an important role in processes like wound healing (3,4), tumorigenesis (5), and angiogenesis (6-8).

Brief Summary Text (27):
The 52 kDa protein receptor of the invention thus is useful for in vitro binding (cell attachment) of the 4N1s and 7N3 peptides as described in application Ser. No. 08/029,333, filed Mar. 5, 1993, now U.S. Pat. No. 5,399,667. The CBD region of TS1 from which these peptides are derived also is implicated in the motility of inflammatory cells and tumor cells. Thus, the receptor is further useful in assay procedures for accessing the processes of inflammation, arthritis and cancer metastasis. This receptor has been found on every cell type so far examined by the inventors. It is on endothelial cells of all types and may provide an anchoring point on TS1, thus allowing the enhancement of the angiogenic functions of other identified peptide regions of TS1.

Drawing Description Text (9):
FIG. 3 shows the effect of enzymes, glycosaminoglycans and divalent metals on labeling of the 52 kDa protein of K562 cells with iodinated 4N1K.

Drawing Description Text (14):
4, chondroitinase ABC 1 unit/ml, 37.degree. C. 90 min;

Drawing Description Text (15):
5, chondroitinase AC 1 unit/ml, 37.degree. C. 90 min;

Detailed Description Text (7):
Treatments of cells with proteases and glycosaminoglycan-degrading enzymes: After washing, 2.times.10.sup.6 cells were resuspended in 200 .mu.l buffer A with or without 1) 20 .mu.g/ml trypsin or chymotrypsin, 2) 1.0 unit/ml chondroitinase ABC or AC, 3) 2.5 units/ml heparinase I, II, or III. The digests were allowed to proceed for 90 min. at 25.degree. C. for proteases and 37.degree. C. for glycosidases (21) respectively. At the end of the incubation, 1 ml of ice-cold buffer B was added to-each reaction and the cells were washed with the same buffer.

Detailed Description Text (35):
To begin determining the properties of this 52 kDa receptor, the sensitivity of the labeled bands to various treatments of the cells was examined. In FIG. 3, lane 1 is control labeling (no treatments), while lanes 2 and 3 show the results of treatment of the cells (prior to labeling) with trypsin and chymotrypsin. Trypsin treatment resulted in a substantial reduction in labeling of the 52 kDa band while chymotrypsin had little effect. Lanes 4 and 5 show results of prior treatment of the cells with chondroitinase ABC and AC respectively. Neither reduced the intensity of labeling or shifted the position of the labeled band.

Detailed Description Text (37):
To determine if the 4N1K peptide was interacting directly with a heparin (heparan) sulfate chain of the 52 kDa protein, cells were treated with heparinase I, II and III singly and in combination before labeling with iodinated 4N1K. None of the enzymes had any effect on either the intensity of labeling of the 52 kDa protein or its mobility

on SDS gels.

Detailed Description Text (95):
35. Yabkowitz. R. and Dixit, V. M. (1991) Cancer Res. 51, 3648-3656